Mag-Bind® Total RNA 96 Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

Mag-Bind® Total RNA 96 Kit allows for rapid and reliable isolation of high-quality total cellular RNA from a wide variety of tissue and cultured cells. Total RNA can be purified from 5-10 mg of tissue or 1 x 10^6 of cultured cells. Purified RNA is suitable for all major downstream applications such as RT-PCR, restriction digestion, and hybridization applications.

If using the Mag-Bind® Total RNA 96 Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Samples are lysed in TRK Lysis Buffer. Proteins are removed with a Proteinase K digestion step. DNA and RNA are then bound to the paramagnetic beads. Genomic DNA is removed with a DNase I digestion step. After two quick wash steps with 70% Ethanol, RNA is eluted.

Since the Mag-Bind® Total RNA 96 Kit uses paramagnetic beads, it can also be adapted to liquid handlers including Beckman Coutler's Biomek FX, Tecan Genesis, Thermo Kingfisher Flex, and Hamilton Star instruments.

New in this Edition:

- Proteinase K is now supplied in a liquid form eliminating the step to respuspend prior to use. Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product Number	M6731-00	M6731-01	M6731-02
Purifications	1 x 96 preps	4 x 96 preps	12 x 96 preps
Mag-Bind® Particles CNR	2.2 mL	8.8 mL	25 mL
TRK Lysis Buffer	50 mL	200 mL	600 mL
VHB Buffer	22 mL	88 mL	3 x 88 mL
RNA Wash Buffer II	50 mL	150 mL	3 x 150 mL
Proteinase K Solution	3 mL	12 mL	60 mL
DNase I	220 µL	4 x 220 µL	12 x 220 µL
DNase I Digestion Buffer	12 mL	48 mL	144 mL
RNA Elution Buffer	15 mL	50 mL	150 mL
User Manual	✓	✓	✓

Storage and Stability

Most components of the Mag-Bind Total RNA Kit are stable for at least 12 months from date of purchase when stored at 22°C-25°C. Mag-Bind Particles Solution should be stored at 2-8°C. Dnase 1 Enzyme should be stored at -20°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer TRK and VHB. Dissolve such deposits by warming the solution at 45°C and gently shaking.

Preparing Reagents

Please take a few minutes to read this manual thoroughly to become familiar with the protocol before beginning the procedure. Prepare all materials required before starting to minimize RNA degradation. Wear gloves/protective goggles and take great care when working with chemicals.

1. Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6731-00	28 mL
M6731-01	112 mL
M6731-02	112 mL per bottle

2. Dilute RNA Wash Buffer II with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6731-00	200 mL per bottle
M6731-01	600 mL per bottle
M6731-02	600 mL per bottle

Mag-Bind® Total RNA 96 Kit Protocol - Tissue

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* with swing-bucket rotor for 96-well plates
- Adapter for 96-well deep-well plates
- Magnetic separation device (Recommend Cat# MSD-01B)
- Equipment for disrupting tissue (MM300 Mixer Mill or Geno/Grinder 2000/2010) or mortar and pestle
- Vortexer
- 100% Ethanol
- Isopropanol
- Multi-channel pipettor and nuclease-free pipette tips
- Sealing film (Recommend Cat# AC1200-01)
- Multi-channel reservoirs (Recommend Cat# AC1331-01)
- 96-well microplates with a minimum capacity of 1 mL (Recommend Cat# SSI-1780-00 or Thermo 96-well deep V microplate for Kingfisher Flex Part No. 95040450)

Before Starting:

- Prepare buffers according to instructions on Page 4.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.

1. Choose one of the two following methods for homogenization of samples:

   A. Manual Sample Preparation
   To prepare samples, collect fresh tissue sample in a 30 mL mortar and freeze by dipping in liquid nitrogen using tweezers or tongs to fill the tube. Grind the tissue using a clean pestle. Transfer the grounded powder and liquid nitrogen into 96-well deep-well plate and allow the liquid nitrogen to evaporate. Add 450 µL TRK Lysis Buffer.

   B. Mechanical Tissue Disruption:
   Place sample into a stainless steel grinding plate with appropriate steel beads. Add 450 µL TRK Lysis Buffer to the samples. Grind sample at 30 Hz for 1-2 minutes according to manufacturer's instructions. For GenoGrinder 2000/2010, grind at 1500 RPM for 2 minutes. Remove the plate from the homogenizer and remove the caps. It may be necessary to centrifuge the plate briefly to remove debris from the caps.

2. Add 20 µL Proteinase K Solution to each sample. Vortex or invert several times to mix the samples.

3. Centrifuge at 4,000 x *g* for 5 minutes.

4. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

   Note: The 96-well plate must have minimum volume of 1.0 mL and be compatible with the magnetic stand used.

5. Add 300 µL 100% ethanol and 20 µL Mag-Bind® Particles CNR to each sample. Vortex for 60 seconds.

   Note: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use. Mag-Bind® Particles CNR and ethanol can be made as a mastermix. Vortexing vigorously for 60 seconds is critical for obtaining good yield.

6. Let sit for 5 minutes at room temperature.

7. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

8. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

9. Remove the plate from the magnetic separation device.

10. Add 400 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

11. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

12. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with ethanol prior to use. Please see Page 4 for instructions.

16. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

17. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

18. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove and residual liquid from the well.

19. Remove the plate from the magnetic separation device.

20. Let sit at room temperature for 2 minutes. While waiting, prepare the DNase I mix.

Component	Amount per Prep	Total Amount per 96-well Plate
DNase Digestion Buffer	98 µL	10.34 mL*
DNase I	2 µL	211 µL*

* A 10% pipetting error has been calculated for a 96-well plate.

21. Add 100 µL DNase I to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

22. Let sit at room temperature for 10 minutes.

23. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with ethanol prior to use. Please see Page 4 for instructions.

24. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

25. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

26. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

27. Remove the plate from the magnetic separation device.

28. Repeat Steps 23-26 for a second RNA Wash Buffer II wash step.

29. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

30. Remove the plate from the magnetic separation device.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove and residual liquid from the well.

31. Add 50-100 µL RNA Elution Buffer to each sample.

32. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for efficient elution.

33. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

34. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

35. Store RNA at -80°C.

Mag-Bind® Total RNA 96 Kit Protocol - Cultured Cells

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 4,000 x *g* with swinging-bucket rotor for 96 well plates
- Adapter for 96-well deep-well plates
- Magnetic separation device (Recommend Cat# MSD-01B)
- Equipment for disrupting tissue (MM300 Mixer Mill or Geno/Grinder 2000/2010) or mortar and pestle
- Vortexer
- 100% Ethanol
- Isopropanol
- Pre-chilled PBS if using cells grown in a monolayer
- Trypsin if using cells grown in a monolayer
- Multi-channel pipettor and nuclease-free pipette tips
- Sealing film (Recommend Cat# AC1200-01)
- Multi-channel reservoirs (Recommend Cat# AC1331-01)
- 96-well microplates with a minimum capacity of 1 mL (Recommend Cat# SSI-1780-00 or Thermo 96-well deep V microplate for Kingfisher Flex Part No. 95040450)

Before Starting:

- Prepare buffers according to instructions on Page 4.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.

1. Harvest cells by choosing one of the following methods (A or B).

   A) For cells grown in suspension:
   1. Determine the number of cells. Do not use more than 1 x 10^6 cells.
   2. Pellet the appropriate number of cells by centrifuging at 500 x *g* for 5 minutes.
   3. Add 450 µL TRK Lysis Buffer and 20 µL Proteinase K Solution to each sample.
   4. Pipet up and down several time to mix the samples.

B) For cells grown in a monolayer:

These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell culture flasks should always be trypsinized.

Direct cell lysis:

1. Determine the number of cells.
2. Aspirate the cell culture medium completely.
3. Add 450 µL TRK Lysis Buffer and 20 µL Proteinase K Solution to each sample.
4. Pipet up and down several time to mix the samples.

Trypsinization of cells:

1. Determine the number of cells.
2. Aspirate the cell culture medium completely.
3. Wash cells with 4°C PBS.
4. Aspirate the PBS.
5. Wash cells with 4°C PBS containing 0.1-0.25% trypsin.
6. Check cells for detachment. Make sure cells are detached before proceeding.
7. Add cell culture medium containing serum to inactivate the trypsin.
8. Transfer cells to an RNase-free microplate.
9. Centrifuge at 500 x *g* for 5 minutes.
10. Aspirate the supernatant completely.
11. Add 450 µL TRK Lysis Buffer and 20 µL Proteinase K Solution to each sample.
12. Pipet up and down several time to mix the samples.

Note: Not removing cell-culture medium completely will inhibit lysis and dilute the lysate. This will affect the conditions for binding of RNA to the Mag-Bind® Particles CNR. Proteinase K Solution and TRK Lysis Buffer can be added as a freshly prepared mastermix.

2. Centrifuge at 4,000 x *g* for 5 minutes.

3. Transfer 400 µL lysate to a 96-well plate. Do not disturb the debris pellet.

Note: The 96-well plate must have minimum volume of 1.0 mL and be compatible with the magnetic stand used.

4. Add 300 µL 100% ethanol and 20 µL Mag-Bind® Particles CNR to each sample. Vortex for 60 seconds.

   Note: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use. Mag-Bind® Particles CNR and ethanol can be made as a mastermix. Vortexing vigorously for 60 seconds is critical for obtaining good yield.

5. Let sit for 5 minutes at room temperature.

6. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

7. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

8. Remove the plate from the magnetic separation device.

9. Add 400 µL VHB Buffer to each sample.

   Note: VHB Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

10. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

11. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

12. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

13. Remove the plate from the magnetic separation device.

14. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with ethanol prior to use. Please see Page 4 for instructions.

15. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

16. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

17. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove and residual liquid from the well.

18. Remove the plate from the magnetic separation device.

19. Let sit at room temperature for 2 minutes. While waiting, prepare the DNase I mix.

Component	Amount per Prep	Total Amount per 96-well Plate
DNase Digestion Buffer	98 µL	10.34 mL*
DNase I	2 µL	211 µL*

* A 10% pipetting error has been calculated for a 96-well plate.

20. Add 100 µL DNase I to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads.

21. Let sit at room temperature for 10 minutes.

22. Add 400 µL RNA Wash Buffer II to each sample.

    Note: RNA Wash Buffer II must be diluted with ethanol prior to use. Please see Page 4 for instructions.

23. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for adequate washing of the Mag-Bind® Particles CNR.

24. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

25. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

26. Remove the plate from the magnetic separation device.

27. Repeat Steps 22-25 for a second RNA Wash Buffer II wash step.

28. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

29. Remove the plate from the magnetic separation device.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove and residual liquid from the well.

30. Add 50-100 µL RNA Elution Buffer to each sample.

31. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

    Note: Complete resuspension is required for efficient elution.

32. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

33. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

34. Store RNA at -80°C.

Troubleshooting Guide

Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you. If for any reason you need further assistance, please contact our technical support staff at our **Toll Free Number 1-800-832-8896.**

Possible Problems and Suggestions

Problem	Likely Cause	Suggestions
Low RNA yields	Incomplete resuspension of Mag-Bind® Particles CNR	Resuspend the Mag-Bind® Particles CNR by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Loss of Mag-Bind® Particles CNR during procedure	Be careful not to remove the Mag-Bind® Particles CNR during the procedure.
	Ethanol was not added to RNA Wash Buffer II	Add ethanol to RNA Wash Buffer II as instructed on Page 4.
	Mag-Bind® Particles CNR not resuspended during binding	Vortex vigorously for 2 minutes after addition of ethanol and Mag-Bind® Particles CNR.
Problem with downstream application	Insufficient RNA was used	RNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature.
Carryover of the Mag-Bind® Particles CNR in the elution	Carryover of the Mag-Bind® Particles CNR in the eluted RNA will not effect downstream applications.	To remove the carryover Mag-Bind® Particles CNR from the eluted RNA, simply place the plate on the magnetic separation device and wait until the eluate has cleared. Carefully transfer the RNA eluate to a new 96-well plate.